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DESICCATED ALLERGENS*

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Since the introduction of smallpox vaccine by Jenner in 1796, rabies vaccine by Pasteur in 1884, and diphtheria antitoxin by Roux and Von Behring in 1893, physicians have been provided with an ever increasing list of "biologicals" for the prevention and treatment of infectious diseases. As the name implies, "biologicals" included all the therapeutic and diagnostic agents derived from living sources, and made up of the physiologically active substances now well known as serums, antitoxins, venoms, toxins, toxoids, vaccines, bacterins, sero-bacterins sensitized bacterial vaccines, allergenic preparations, glandular substances, hormones, natural vitamins, tissue extracts, body fluids, organic salts, hemopoietic substances and others.

There has been a never ending research to increase the stability and preserve the biological activity of therapeutically valuable sub-

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stances. This biological activity is associated with colloidal aggregates such as proteins, glucoproteins and glucolipoids, but these aggregates are of colloidal dimensions and are of great chemical complexity. Colloids are characteristically unstable because the colloidal dispersion is easily influenced by natural physical factors. Serum, for example, is a colloidal solution of the blood proteins which gradually and continuously undergoes changes under the influence of heat, light, and electric charges. If these changes are permitted to proceed unchecked, the altered colloidal state will be manifest by changes in pH, color, surface tension, electrical conductivity, viscosity and clarity. It is commonly known that the aging of serums in the liquid state causes a change of color and the appearance of a deposit of salts and finely divided amorphous material. As early as 1896 C. J. Martin suggested the possibility of preserving the biologic potency of guinea pig complement by drying it. Noguchi actually accomplished this in 1907 by drying amboceptor on filter paper. In 1909 Shackell went a step farther by freezing complement and then drying it using a desiccant. This was a long drawn out process but produced complement which would remain potent for several months.

The above methods, while producing complement which did not deteriorate as soon as a fresh complement, were far from perfect. In April, 1931, Reichel of Mulford Biological laboratory presented the "Lyophile Process" of preserving biological substance by means of rapid freezing and rapid dehydration under high vacuum. This definiton emphasizes the rapidity of the process and accounts for the modern successful processing of biologicals for the preservation of their potency. The rapidity of this process is accomplished by the use of dry-ice to freeze the substance and the use of vacuum pump to dehydrate the frozen material, the water vapor being absorbed in a low temperature condenser. This method can of course be applied to any biological. The resulting substance is a porous product which redissolves with remarkable ease and completeness on the addition of distilled H_2O. Reichel used the term "lyophile" to describe the process because it means "solvent-loving." The lyophile substance will keep indefinitely and the redissolved product will resemble in all biological respects the original substance because our procedure of dehydration. (Figure 2.)

In 1938, Flosdorf and Mudd described an improved procedure and apparatus for the preservation of biologic substances and they termed this method the "Cryochem." This term was derived from the Greek to denote desiccation in vacuo from the frozen state plus the use of a chemical desiccant. This use of a chemical desiccant is the essential difference between the lyophile and the cryochem processes. The vacuum pump itself is not adequate to remove all the water vapor from the frozen substances. In the lyophile apparatus a low temperature condenser is used to supply the extra factor for this purpose. In the cryochem apparatus, a desiccant such as calcium sulfate is used for this purpose. As a matter of fact, if the vacuum pump is of sufficient power and the desiccant sufficient to absorb moisture at an efficient rate, the substance being dried will automatically be maintained in a frozen state, consequently dry-ice is not necessary and this eliminates one of the objections to the lyophile apparatus as dry-ice is expensive. The calcium sulphate which is ordinarly used can be regenerated and used 18 to 20 times, that is, the moisture can be driven off by dry heat. At the Veterans Administration Facility, Pittsburgh, Pa., we use the cryochem apparatus and regenerate the calcium sulphate in a large bakers oven. If desired, the process of cryocheming can be accelerated by using ordinary ice from a refrigerator around the substance being cryochemed, but this is not necessary under ordinary circumstances. The use of small amounts of biologicals to be run through in small vials, the vacuum pressure is apt to draw off the liquid and it is therefore desirable in these cases to pre-freeze in the freezing compartment of a refrigerator.

The amount of the desiccant used determines the total volume which may be dried before regeneration of the desiccant becomes necessary. For each one hundred pounds of desiccant 2,000 cc. of liquid can be dried. However, it has been found that the life of the calcium sulfate is lengthened if not more than ¼ of its total capacity is used in a single run. We have found that a single run of 1500 cc. can be made. But a greater amount of time is required for the remaining 500 cc. to be dried. The cost of electricity for running the cryochem machine at our hospital has been estimated as one cent per hour.

Technic of Cryochem

The cryochem consists of a chamber for the desiccant, a vacuum pump and gauge and a manifold for connections to the containers. Perhaps those not familiar with the machine would be interested in our procedure of dehydration. (Picture.)

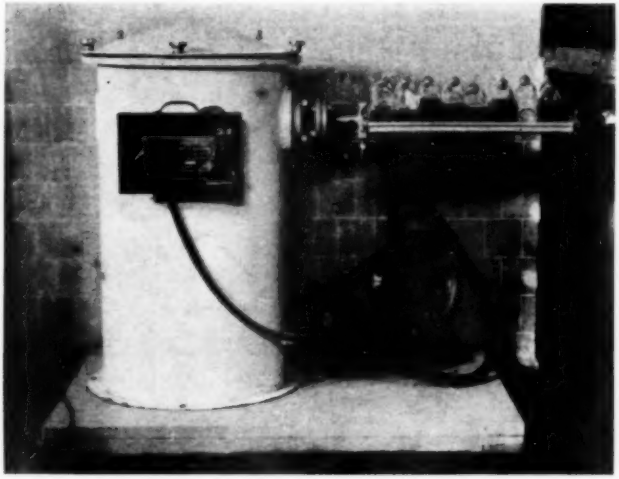
After the machine is tested for vacuum tightness the containers are filled and attached to the manifold and allowed to rest on the freezing tray at a 45 degree angle. Frequently, but not always, we pack these containers in ice (from the refrigerator) to speed the degassing and freezing. This is not a necessary step as freezing will take place when the substance has been degassed and a complete vacuum is reached. After the substances are degassed and frozen the tray is raised to a horizontal position to allow a wider surface for drying. When the air in the machine has been adequately removed and the gauge indicates 600 to 700 microns the ideal vacuum pressure has been reached. The smybol μ (micron) is used, as is customary in high vacuum work, to designate a pressure of 0.001 mm. of Hg. The process is then allowed to proceed until the material is completely dry. The time required for desiccation depends on the quantity and nature of material.

At the end of the process the containers must be removed aseptically. Dry air is slowly admitted by syringe at the exhaust tubes of the containers. The air is made dry by being forced through calcium sulfate filter in the syringe. Immediately the containers are detached, re-evacuated and sealed. The containers are then tested for leakage with a spark coil vacuum tester (on 110 V. lighting circuit A.C.). Bottles with rubber stoppers are dipped in paraffin to aid the preservation of rubber. Labels are checked and recorded indicating original volume and the allergens stored in their respective places. When the materials are needed the desired amount of sterile H_2O is added to make the original volume. This is indicated on the label. These substances are easily and rapidly dissolved. Cultures for sterility are made on all bottles.

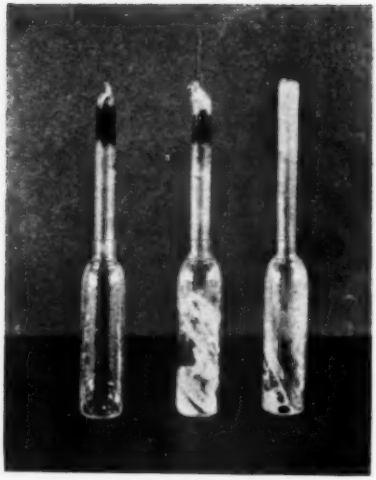
Purpose of Investigation

As allergy center for the Veterans Administration, we prepare and dispense allergens of all types for skin tests and treatments to

1. Manifold holding bottles being desiccated.



2. Cryochem Unit.



3. Desiccated Blood Serum (5 c.c.) used for passive transfer.

some ninety other facilities. Because of the large amounts of pollens necessary, our manufacturing process must be carried on all year through by extracting various pollens, foods and other substances. The seasonal substances must naturally be extracted in season. Frequently we must keep on hand allergens for use in widely separated parts of the country. All this entails practically a continual process of manufacture. Many of the pollens are very expensive and difficult to obtain. We must also keep on hand a large supply of expensive allergens which are called for only periodically. Obviously, the problem of storage and the preservation of allergens for a long period of time is a very important one to us. We have long been in the habit of storing glycerinized allergens in ice boxes. This has entailed a very great amount of space and large number of refrigerators. It was thought that it would be possible to preserve these biologicals for indefinite periods in a cryochemed state and for this reason the cryochem apparatus was purchased. The purpose of our investigation was to check the potency of cryochemed pollen and other extracts against the glycerinized pollen and other extracts against the glycerinized pollen and other extracts after varying periods of time.

Method of Investigation

By Coco's technic 40 various pollens were extracted at a 6% concentration. A nitrogen content determination was made on each pollen extract. To one-half of the quantity of each pollen extract glycerine was added and stored in the refrigerator in accordance with our routine procedure. The other half of each pollen extract was divided into 6 groups of 1 oz. quantities in pyrex bottles. These bottles were prepared and stored in the following manner:

1. Pollen extract and no glycerine (plain)—stored at Rm. temp.
2. Pollen extract and no glycerine (plain)—stored at Rfg. temp.
3. Glycerine extract — Stored at room temperature.
4. Glycerine extract — Stored at refrigerator temp.
5. Cryochemed extract — Stored at room temperature.
6. Cryochemed extract — Stored at refrigerator temp.

At the end of 6 months a check on the nitrogen was made on each bottle. After 1 year storage another nitrogen determination was made. Nitrogen content alone does not determine the potency of an allergen. The surest test of the potency is a passive transfer. This consists of the intradermal injection of blood serum from a known positive allergic case into the arm of a non-allergic person. This renders the local site allergic to whatever specific allergen is involved. After 24 hours (incubation period) the specific allergen itself is injected into the same site and if the allergen is still potent a typcal reaction of sensitivity should develop.

Because non-allergic patients could not be used as donors for passive transfers, this phase of investigation was stopped at our hospital after 6 months of investigation. However our consultant, Dr. L. H. Criep, would take the material prepared at our hospital to one of the Pittsburgh Hospital Clinics and test the allergen potency by passive transfers there.

Also for one year skin tests were run routinely in our clinic using our regular, freshly prepared allergens and the cryochemed extract. These tests were run parallel using the same amount of each dilution in both tests. This was done to avoid error in varying amounts in the test which might give false positives.

Results

After 6 months there was a slight drop in the plain extract kept at room temperature. The cryochemed extract and glycerine extract gave the same result on skin test of 0.01 N. on clinic patients. After 1 year the only change in the Nitrogen was a drop in the plain extract stored at room temperature. The skin tests on plain extract at room temperature were weaker than the glycerine or cryochemed extracts. As the charts will show, by passive transfer there is no loss of potency in the cryochemed allergens after 6 months' storage in the refrigerator or at room temperature. As you will note the glycerine extract gave good results but after a year glycerine extracts even though they are stored in the refrigerator, lose some of their original potency. Nitrogens on glycerinated extracts are difficult to determine, and our comment is made on observation of old extracts used on known positive cases. As to the parallel skin test run in our clinic of the cryochemed extracts

against our regular freshly prepared allergens, there was a definite uniformity in the results. These tests were limited chiefly to food and inhalant allergens and the pollens typical of this district.

Comment

We observed that foods of high sugar content would often produce a thick, sticky appearing mass instead of the light, porous, frothy material as found in the others.

Bacterial cultures were cryochemed to keep up our stock supply. Stock cultures so prepared not only remained viable for years but their antigenicity and virulence did not deteriorate.

Wassermann Complement was checked repeatedly. When there was an excess of strong complement in the laboratory it was desiccated. The titration was marked on the vial and date of desiccation noted. These were checked at the end of five days, two weeks, one month, three months, six months, one year and eighteen months. In all the specimens were found a marked decrease in the titration. Many were even too low to be used. It has been reported that the stabilization of high vacuum dried guinea pig complement requires the addition of sodium acetate. Others have investigated this same problem with successful finding. We will endeavor to study this further in the future.

Desiccated allergens remain sterile and retain their potency for a longer time than liquid material. They have a greatly extended period of usefulness. Food allergens do not require refrigeration for storage, and save storage space. Skin tests on cryochemed allergens 18 months old gave the same result as freshly prepared extracts. Desiccated allergens maintain unaltered the physical structure and colloidal properties of the original material and permit the carry over of labile agents from one season to another with little or no loss of efficiency.

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PASSIVE TRANSFER AT 6 MONTHS

	Allergen	Stored At	Reaction
Donor No. 1 J.A.—30-215	Ragweed 0.01 (control)		Negative
Patients Serum			
0.1 c.c.	Ragweed 0.01 (glyc)	Rfg. temp.	2 plus
0.1 c.c.	Ragweed 0.01 (plain)	Rm. temp.	plus-minus
0.1 c.c.	Ragweed 0.01 (plain)	Rfg. temp.	1 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rfg. temp.	2 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rm. temp.	2 plus
Donor No. 2 S.D.—48-120	2 Ragweed 0.01 (control)		Negative
Patients Serum			
0.1 c.c.	Ragweed 0.01 (glyc)	Rfg. temp.	2 plus
0.1 c.c.	Ragweed 0.01 (plain)	Rm. temp.	minus
0.1 c.c.	Ragweed 0.01 (plain)	Rfg. temp.	1 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rfg. temp.	2 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rm. temp.	2 plus
Donor No. 3 R.P.—50-386	2 Ragweed 0.01 (control)		Negative
Patients Serum			
0.1 c.c.	Ragweed 0.01 (glyc)	Rfg. temp.	2 plus
0.1 c.c.	Ragweed 0.01 (plain)	Rm. temp.	minus
0.1 c.c.	Ragweed 0.01 (plain)	Rfg. temp.	1 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rfg. temp.	2 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rm. temp.	2 plus

PASSIVE TRANSFER AT 6 MONTHS

	Allergen	Stored At	Reaction
Donor No. 4 C.H. Emp. Patients Serum	2 Ragweed 0.01 (control)		Negative
0.1 c.c.	Ragweed 0.01 (glyc)	Rfg. temp.	3 plus
0.1 c.c.	Ragweed 0.01 (plain)	Rm. temp.	1 plus
0.1 c.c.	Ragweed 0.01 (plain)	Rfg. temp.	2 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rfg. temp.	3 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rm. temp.	2 plus
Donor No. 5 K.A. Emp. Patients Serum	2 Ragweed 0.01 (control)		Negative
0.1 c.c.	Ragweed 0.01 (glyc)	Rfg. temp.	3 plus
0.1 c.c.	Ragweed 0.01 (plain)	Rm. temp.	1 plus
0.1 c.c.	Ragweed 0.01 (plain)	Rfg. temp.	2 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rfg. temp.	3 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rm. temp.	3 plus
Donor No. 6 K.E. Emp. Patients Serum	2 Ragweed 0.01 (control)		Negative
0.1 c.c.	Ragweed 0.01 (glyc)	Rfg. temp.	3 plus
0.1 c.c.	Ragweed 0.01 (plain)	Rm. temp.	1 plus
0.1 c.c.	Ragweed 0.01 (plain)	Rfg. temp.	2 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rfg. temp.	3 plus
0.1 c.c.	Ragweed 0.01 (Cryo)	Rm. temp.	3 plus

NITROGEN CHART

ALLERGEN	Temp. Stored	N—6 months	N—1 year	N—18 months
Ragweed 1.5 N. (plain)	Rm. temp.	1.0 N.	0.8 N.	0.7 N
Ragweed 1.5 N. (plain)	Rfg. temp.	1.2 N.	0.9 N.	0.9 N.
Ragweed 1.5 N. (glyc)	Rm. temp.	1.1 N.	0.9 N.	0.75N.
Ragweed 0.9 N. (glyc)	Rfg. temp.	0.9 N.	0.9 N.	0.9 N.
Ragweed 1.5 N. (Cryo)	Rm. temp.	1.5 N.	1.48 N.	1.4 N.
Ragweed 1.5 N. (Cryo)	Rfg. temp.	1.5 N.	1.4 N.	1.4 N.
Timothy 0.62N. (glyc)	Rfg. temp.	0.62N.	0.62 N	0.62N.
Timothy (glyc)	Rm. temp.	0.76N.	.7 N.	0.7 N.
Timothy 1.5 N. (plain)	Rfg. temp.	1.3 N.	.78 N.	0.72N.
Timothy 1.5 N. (plain)	Rm. temp.	.92N.	.76 N.	0.70N.
Timothy 1.5 N. (Cryo)	Rfg. temp.	1.5 N.	1.5 N.	1.43N.
Timothy 1.5 N. (Cryo)	Rm. temp.	1.5 N.	1.42 N.	1.41N.
Sagebush 0.76N. (glyc)	Rfg. temp.	.75N.	.75 N.	.74N.
Sagebush .76N. (glyc)	Rm. temp.	.70N.	.68 N.	.64N.
Sagebush 1.52N. (plain)	Rfg. temp.	.86N.	.83 N.	.75N.
Sagebush 1.52N. (plain)	Rm. temp.	.86N.	.79 N.	.72N.
Sagebush 0.76N. (Cryo)	Rfg. temp.	.76N.	.76 N.	.75N.
Sagebush 0.76N. (Cryo)	Rm. temp.	0.76N.	.75 N.	.75N.
Plantain 0.4 N. (Cryo)	Rm. temp.	0.4 N.	0.386N.	0.38N.
Plantain 0.4 N. (Cryo)	Rfg. temp.	0.4 N.	0.39 N.	0.39N.

NITROGEN CHART

ALLERGEN STORED AT ROOM TEMPERATURE		1 YEAR
Rye grass	1.2 N.	1.2 N.
Blue grass	0.8 N.	.8 N.
Box Elder	0.8 N.	.79 N.
Roses	1.2 N.	1.2 N.
Cheat grass	1.5 N.	1.5 N.
Desert grass	0.8 N.	.8 N.
Spear grass	1.0 N.	.98 N.
Careless Weed	0.21N.	.2 N.
Spreading Amaranth	0.9 N.	.87 N.
Broncho grass	0.9 N.	.9 N.
Mustard	1.4 N.	1.35 N.
Canary grass	1.3 N.	1.3 N.
Coastal Lens Scale	0.6 N.	.6 N.
Callopsis	1.1 N.	1.0 N.
Englemans Oak	0.14N.	.14 N.
Lambs Quarters	1.1 N.	1.1 N.
Elm Tree	3.3 N.	3.2 N.
Darnell grass	0.48N.	.47 N.
Calif. Bl. Oak	2.0 N.	2.0 N.
Ash Tree	1.2 N.	1.2 N.
Winged Ragweed	0.5 N.	.5 N.
Perennial Rye	0.4 N.	.39 N.
Avena Barvata	1.0 N.	1.1 N.
Bract. Scale	1.1 N.	1.0 N.
Big cane spruce	0.8 N.	.8 N.
English Walnut	1.2 N.	1.18 N.
Lens Scale	0.7 N.	0.7 N.
Giant Bramegrass	1.0 N.	1.0 N.
Rough Pigweed	0.5 N.	.49 N.
Alfalfa	1.6 N.	1.58 N.
Canary's Dock	0.8 N.	0.8 N.
Freemont's Cottonwood	.7 N.	.7 N.
Red Scale	0.15N.	.146N.
Atriplex Canescen	1.0 N.	1.0 N.
Bract Scale	1.1 N.	1.0 N.
Cottonwood	0.7 N.	0.67 N.
Darnell grass	0.48N.	0.47 N.
Desert Ragweed	0.8 N.	0.76 N.
Mountain Cedar	.1 N.	.1 N.
Marsh Elder	0.34N.	.33 N.
Orchard grass	0.17N.	.16 N.
Ray-Rye Grass	1.2 N.	1.16 N.
Rough Pigweed	0.5 N.	0.5 N.
Sheep Sorrell	.36N.	.35 N.
Wild Rye	.28N.	.27 N.
Wild Oats	.72N.	0.69 N.

NOTES ON THE TECHNIC OF THE KOLMER COMPLEMENT FIXATION TESTS FOR SYPHILIS*

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The technic of the Kolmer complement fixation tests for syphilis, as published about twenty years ago (1), was based upon such thorough and extensive fundamental studies in all phases of the Wassermann test (2) that no important changes have been since indicated or required insofar at least as the basic principles are concerned.

At that time Kolmer described quantitative and qualitative procedures for testing both serums and spinal fluids. In 1935 he changed the doses of serum to larger amounts in both tests (3) and in 1939 further simplified the qualitative test to enable its conduct with two instead of three tubes which, in the various Serologic Surveys, has been found to be equally specific and almost as sensitive as the quantitative or six-tube test. Still more recently, he has described methods for conducting the quantitative and simplified tests with both serums and spinal fluids using one-fifth amounts of all reagents (5). These procedures require the use of test tubes of about the same size as employed in the Kahn test but otherwise the technic is exactly the same as in the regular procedures, except that one-fifth amounts of serum, spinal fluid, antigen, complement, hemolysin and sheep corpuscle suspensions are employed. Since the results of comparative tests with the regular procedures have yielded practically identical results in regard to both sensitivity and specificity, it is apparent that the economy effected insofar as the reagents are concerned greatly facilitates the routine use of a complement fixation test in the serological diagnosis of syphilis. For example, 17 to 18 cc. of complement is ordinarily required for the conduct of

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100 regular quantitative tests but only 5 cc. by the one-fifth technic. And whereas 6 cc. is ordinarily required for the conduct of 100 regular simplified tests, only 2 cc. is required by the one-fifth technic, or about 10 cc. for 500 tests. Furthermore, while 1 cc. of antigen is ordinarily required for the conduct of 240 regular quantitative or 1200 simplified tests, this amount ordinarily suffices for the conduct of 1200 quantitative or 6000 simplified tests by the one-fifth technic. There is, however, no reduction in the amount of time and skill required as otherwise the technic is exactly the same as that of the regular procedures. Indeed, even greater care is required as the amounts of reagents are reduced to volumes of 0.1 or 0.2 cc. whereas one of the important principles incorporated into the technic of the regular procedures has been the use of reagents diluted to volumes of 0.5 or 1.0 cc. in order to reduce to a minimum errors in pipetting. Furthermore, since the total volume of the one-fifth method is 0.7 cc. instead of 3 cc., as in the regular methods, the reading of positive reactions is not quite as easy as in the latter and especially the finer distinctions of positive reactions as differentiation between + and ++ or between ++ and +++ but, after all, this does not sufficiently interfere with or reduce the accuracy of readings from the practical standpoint.

With either the regular or one-fifth quantitative and simplified tests, the reactions observed in the respective tubes may be recorded as ++++, +++, ++, +, ± or — and the results reported as positive, doubtful or negative, as recommended by the American Committee on Evaluation of Serodiagnostic Tests for Syphilis of the United States Public Health Service cooperating with the American Society of Clinical Pathologists although Kolmer (6) recommends reporting reactions as strongly positive, weakly positive, doubtful or negative.

Insofar as a choice between quantitative and simplified tests, by either the regular or one-fifth methods, is concerned, the former is preferred because somewhat more sensitive, since natural antisheep hemolysin is removed from sera by absorption with sheep corpuscles, but experience has shown that the simplified tests by either method are just about as sensitive insofar as positive reactions are concerned and equally specific. Under the conditions the regular or one-fifth simplified tests are as satisfactory and acceptable as the quantitative

procedures and needless to state are preferred to the latter in those laboratories required to conduct large numbers of tests. Without doubt, however, the quantitative tests are preferred, whenever possible, in the testing of the serums or spinal fluids of cases of syphilis under treatment since the results are of more value to the physician in relation to serologic improvement or "fastness" than the simplified tests.

Kolmer has also described an improvement of his C.L. antigen by reenforcing it with acetone insoluble lipoids (7, 8). This new antigen is preferred because of increased sensitivity, while preserving its remarkable specificity, but experience has shown that either the old or new antigens may be employed with satisfactory results. Incidentally, it is important, however, to keep the extracts tightly stoppered at room temperature instead of in a refrigerator in order to prevent the precipitation of cholesterol and other antigenic lipoids at the latter temperature. Under these conditions the antigens keep remarkably well over periods of a year or longer. Changes in antigenic sensitivity and anticomplementary activity rarely occur although Kolmer advises re-titration every six months for the detection of possible deterioration.

Kolmer (9) has likewise approved and adopted an optional or alternative method for determining the optimum dose of antigen to use in his tests, founded upon the technic described by Boerner and Lukens (10), since the latter is based upon sound principles, although a decision upon the optimum dose to employ by this method of titration is sometimes more difficult than with the method originally described by him.

Since an important principle of the Kolmer complement fixation tests is to use the minimum amount of complement for the purpose of securing reactions with the maximum of sensitivity consistent with specificity, difficulties are sometimes encountered by reason of prezone reactions and especially in tests employing spinal fluids. According to the Kolmer method of titration the complement is used in a dose corresponding to two full units. It sometimes happens, however, that negative or normal sera tested by the quantitative methods give reactions like -1-3-4 with complete hemolysis of the serum and antigen controls although such prezone reactions are

rarely, if ever, observed with normal sera in the simplified tests. In quantitative tests employing normal spinal fluids, however, reactions like 44444 or of lesser degree may occur with complete hemolysis of the fluid and antigen controls. Similar nonspecific reactions may be observed in the simplified tests with normal or negative spinal fluids. Furthermore, in both the quantitative and simplified tests with normal sera the antigen controls may sometimes show complete or partial inhibition of hemolysis without such prezone reactions occurring in tubes carrying serum and antigen and with complete hemolysis of the serum controls.

Inexperienced serologists are always likely to jump to the conclusion that the hemolysin or antigen is responsible. But since anti-sheep hemolysin preserved with glycerol keeps almost indefinitely in the refrigerator, and the antigen kept at room temperature rarely, if ever, acquires increased anticomplementary properties for six months or longer, these reagents are rarely responsible. Indeed, it is now known that the complement sera of some guinea pigs is at fault.

The mechanism of these nonspecific reactions is unknown. Giordano and Carlson (11) have thought that the sera of some guinea pigs contain a substance which fixes complement in the presence of antigen at refrigerator temperature and since this is not detected in the titrations of complement and hemolysin conducted at 37° C. suggested that each guinea pig serum employed as complement be subjected to pre-testing at refrigerator temperature. Apparently, however, it is due to the fact that the complement sera of some guinea pigs are peculiarly, and for reasons as yet unknown, susceptible to inactivation by antigen due to the absence of sufficient amounts of protective proteins in spinal fluids and the higher dilutions of serum carried in the last three or four tubes of the quantitative serum tests (12). At least they occur only in the quantitative but not in the simplified tests employing sera. But since spinal fluid contains very much less protein than serum, they are especially likely to occur in both quantitative and simplified tests employing spinal fluids as well as, occasionally, in antigen controls which carry neither patient's serum nor spinal fluid.

At least this explanation of their mechanism is supported by the fact that the addition of egg-albumin to quantitative tests with serum

and to both quantitative and simplified tests with spinal fluid prevents their occurrence in the great majority of cases (13, 14). Consequently Kolmer advises the use of egg albumin-complement mixtures routinely in all quantitative and simplified tests employing spinal fluid (15). Likewise in quantitative serum tests if and when prezone reactions are being observed, which is especially likely to be the case during the warmer months of the year, although, as previously stated, they are not required in simplified tests employing sera.

While the use of egg albumin-complement mixtures under these circumstances may slightly reduce the sensitivity of reactions, the results of comparative tests conducted with plain complement have not shown any reduction in the incidence of positive reactions with syphilic sera and spinal fluids. Furthermore, with their use Kolmer does not believe it necessary to pre-test guinea pig complement and does not do so in his own laboratory (15).

Pooled complement preserved by the lyophile or cryochem processes may be employed in the conduct of the Kolmer tests and is used routinely without pre-testing in the Pennsylvania State laboratories. They have the great advantage of pooling the sera of large numbers of guinea pigs as well as that of convenience although, as pointed out by Kolmer, Richter and Lynch (16), it is inadvisable to use any lot of such preserved complement after ten months because of the chances of some gradual deterioration in fixability by antigen and syphilis reagin even though hemolytic activity is satisfactory.

Kolmer (15) also advises that during the secondary incubation to watch the serum, antigen and hemolytic system controls and 10 minutes after these have shown complete hemolysis (which is usually 25 to 30 minutes after the tests have been placed in the water bath at 37° C.) to remove the racks and make the readings at once. This materially increases the sensitivity of the reactions and is recommended as a routine in the case of experienced serologists. In the case, however, of those tests in which the controls are not completely hemolyzed, the incubation should be continued for a total of one hour, which frequently permits the reading and interpretation of reactions with sera and spinal fluids which are slightly anticomplementary.

Otherwise only a few minor details may be worthy of mentioning. Kolmer recommends the preparation of saline solution with chemically pure sodium chloride and freshly distilled water (17). He also advocates adding 0.1 gm. magnesium sulfate to each 1000 cc. routinely and especially in those areas where the water employed has been found to render saline solution somewhat anticomplementary. Test tubes for the regular quantitative and simplified tests should have an inside diameter of 8.5 × 1.5 cm. and for the one-fifth methods 7.5 × 1.0 cm., but tubes with outside dimensions of these sizes or otherwise slightly larger or smaller, may be employed as slight differences in size are of no importance. But, in conclusion, I would be remiss if I failed to emphasize how anxious Dr. Kolmer is to have his tests performed exactly as he has described them in all details regardless of how trivial and unimportant they may appear. Having had the advantage of his training, I well realize how careful and punctilious he is in technic and the United States Public Health Service lays great stress on the need and advisability of all medical technologists closely adhering to the technics of all serologic methods exactly as described by their authors.

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PRACTICAL POINTS ON PLASMODIUM IDENTIFICATION*

By JOHN J. ANDUJAR, M.D.

Introduction

The present world crisis again turns the spotlight on Malaria, the international scourge. We read in the papers how malaria so weakened and decimated our heroes of Bataan and Corregidor that further active resistance became impossible. Looking into the future we face a world where distance is no longer a major factor, and intercontinental travel will bring home to us diseases alien to our land. With great numbers of our fellow citizens from the north assigned to southern stations in areas where malaria is endemic, it is to be expected that medical technologists not previously conversant with Plasmodium will shortly have opportunities to become acquainted with this parasite. Obviously this work cannot supply the information contained in the mountainous tomes written on the subject. Our purpose is simply to emphasize several important and highly practical points which should be of service in striking up this new acquaintanceship.

Definition

The Plasmodia, cause of malaria, are ameboid protozoa parasitic in and upon red cells and able to reproduce asexually (schizogony) in man and sexually (sporogony) in the definitive host, the mosquito. In man they consume and destroy the erythrocytes, leaving the product of their metabolism, the melanin like substance called malarial pigment.

Basic Facts

The infective Anopheles mosquito initiates the asexual cycle by

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depositing sporozoites through a bite. These immediately begin the process of growth and reproduction known as incubation and consuming one to two weeks. Any attempt to identify plasmodia in this period in the circulating blood is quite futile, but the medical technologist is spared this effort since the patient is quite symptom free until enough parasites have been formed to make a concentration of about one plasmode to each 100,000 RBC. The very recent brilliant work of Manwell and Goldstein[1] as well as Taliaferro, Cannon and others clearly indicates that our knowledge of the asexual cycle is only partial, and that this incubation period is purely exo-erythrocytic, that is it takes place in fixed cells of the reticulo-endothelial and lymphoid series rather than in mature erythrocytes. The young schizonts, however, eventually enter immature red cells and progress through the signet ring and ameboid stages to the large merocyte or rosette. The latter bursts into its component 8 to 30 merozoites, which in turn repeat the cycle. After some weeks, or even in a few days, sexual forms begin to appear in the blood, but they do not reproduce until sucked up into the stomach of the Anopheles mosquito. Here they form thousands of new sporozoites which migrate to the salivary glands to await the opportunity of infecting the next individual to suffer a bite. To a great extent the incubation period then resulting will depend on the number of sporozoites deposited in the bite.

At least three divisions of the genus Plasmodium are recognized: the avian, which includes such species as P. cathemerium, gallinaceum, relictum; the simian, including P. knowlesi and cynomolgi; and the human, which includes P. vivax, falciparum, ovale and malariae. P. ovale is practically unknown in this country, although first described by Craig in 1900. It causes a relatively self limiting, mild form of tertian malaria. It may easily be so new to man that it has not gained virulence. At the other extreme is P. malariae, cause of quartan fever. Formerly quite prevalent throughout the world, it is now more and more limited and in the U. S. is decidedly uncommon. Even in tropical areas of this hemisphere where we had observed it to be endemic it is becoming quite unusual, so that we may hope that this Plasmodium is ending its phase of virulence to man. We are therefore chiefly concerned in this paper with P. vivax of tertian malaria and P. falciparum of estivo-

autumnal (malignant tertian) malaria. Estivo-autumnal malaria does not exhibit the clock like regularity of the 72 hour chill of quartan nor the 48 hour chill of tertian fever. It is far and away the most freakish and the most severe. For some strange reason the plasmodia leave the circulating blood just after the signet ring stage and complete their growth in capillaries where they obstruct and cause considerable damage.

Malaria in the Laboratory

Many procedures have been propounded to enable the medical technologist to assist in the diagnosis of malaria. In 1927 Henry[2] of France proposed the melano-flocculation and ferro-flocculation tests on the patient's serum. Only the former survives and there is marked disagreement as to its value. Complement fixation tests have been hampered until recently by the lack of a specific antigenic principle, but may soon offer some promise. The Proske and Watson[3] protein-tyrosin test determines the tyrosin index of the patient's serum euglobulin. In malaria, the figure is several times normal. While the test is easily performed in the average laboratory, there is not full accord as to the value or interpretation of the procedure. Skin tests, whether using digested plasmodia or aqueous extracts of malarial blood clots, have been disappointing. Precipitin tests with infected placental blood are also of questionable worth. Indirectly the Wassermann test has been studied numerous times in relation to malaria. Kitchen et al.[4] found that acute malaria caused a very high proportion of positive Wassermann tests in non-syphilitics.

To make a long story short, however, we may say that of the numerous laboratory devices proposed, only two have stood the test of time: one is the recognition of the malarial pigment ingested into wandering cells, and this requires a highly expert malariologist; the other is the universal thick or thin film of the circulating blood to demonstrate Plasmodia.

Practical Points in Plasmodium Identification

1. It is startling to note how much misinformation exists as to when blood should be collected for the plasmodial search. Some physicians seem to be firmly convinced that the smears should be

made "at the height of the chill." Many are the sleepy eyed technologists who have been aroused at 3 A.M. because Mrs. Blank was having her chill. In actuality, the time of collection is not important so long as you AVOID the chill. Since it is the rupture of the thousands of erythrocytes from the bursting merocyte that precipitates the chill, all one could see at this time in the blood would be the tiny merozoites, probably the most difficult to identify. As a matter of fact, two smears a day for two or three days will most certainly reveal the plasmodia if they are present. Possibly the best time for a single smear is about 6 hours after the chill but if negative the procedure just mentioned should be instituted.

2. Immediately the subject of concentration arises. Any good procedure, such as that of Bass and Johns[5] will most certainly be tedious and in acute malaria quite unnecessary. But in the chromic or latent infections where the ordinary finger drop smears are repeatedly negative, these concentration methods are not excelled. That previously referred to will increase your chances of finding a plasmodium by about 900 times, and is given in detail by most standard texts.

3. The decision as to whether a thick drop or a thin film should be made is not really a difficult decision. For safety's sake both types should always be prepared on every examination, and can readily be placed on the same slide if desired. Undoubtedly, the novice will encounter great difficulty in recognizing distorted or half hidden plasmodia under several layers of intact or even lysed erythrocytes. It behooves the beginner to search many good fields of a good smear of usual routine differential thickness. If a 5x ocular or a 60x oil immersion objective is used, the field will be larger and brighter so that more cells can be covered. The individual cell under suspicion can be checked under the usual 10x ocular or 97x lens. Even the expert malariologist will be inclined to check all thick drop preparations with good thin films in order to classify the parasite.

4. The question of stain is of some importance. In the case of thick drop smears any good standard technique for lysis of erythrocytes and slight overstaining with Wright-Giemsa such as detailed by Wilcox and Logan[6] should most certainly be employed.

The ordinary Wright or Feemster stains for routine differential are quite satisfactory for thin films. It should be noted that no stain at all, that is, a fresh preparation, is most fascinating when the ameboid phase is reached. Particularly is this so if a small inexpensive disc of polaroid is placed in the condenser of the microscope and another similar disc in the platform of the ocular. The pigment granules, being doubly refractile, show up beautifully. Vital stains have an especially beautiful selective affinity so that all manner of color combinations is possible. None take the place of the fixed smear, however.

5. In our experience, easily the commonest error among novices is the mistaking of platelets for plasmodia. It should be remembered that the malarial plasmode is always in or on an erythrocyte when in the circulating blood. The red cell may be nearly destroyed, but its ghost will still be there. Therefore if the "plasmodia" are found all over the slide, both on and off red cells, the chances are good that they are platelets. Far more important is the fact that platelets almost invariably show a pale halo about their nuclear material whereas no such ring is present about the cytoplasm of the plasmode. Of course, the chromatin dot of malarial parasites is not present, if the structure is a thrombocyte.

6. While the chief aim of the technologist is of course to find the plasmodia it should be kept in mind that the classification of the species is of some importance. In the average case this presents no difficulty to the experienced worker. Knowing that malarial drugs such as quinine, atabrine, tebetren and plasmochin all have different actions on different phases, the malariologist has learned the salient points of differentiation in order to facilitate individualized treatment. It is not within our scope to review this problem in any detail, but we may at least recall certain facts. P. falciparum, for example, leaves the circulating blood just after the young signet ring stage. Therefore, if ameboid forms are seen swelling a pale cell, the plasmodium cannot obviously be falciparum. Again, it reappears in the sexual or gametocyte phase. These are the highly characteristic crescents that cannot be mistaken for any other plasmodium. Again a word of caution against confusing these structures with immature, large platelet fragments is in order. Again we must

recall that so called applique or scalloped forms are a characteristic. The P. falciparum in the young form will attack and dent the edge of the erythrocyte, and in fact may remain in this projecting or overhanging position giving the usually convex rim of the red cell a decidedly concave curve. The P. malariae of quartan fever has the very unusual banded or ribbon forms and is characterized positively by the fact that it is the only plasmodium having only 8 instead of 15 to 30 merozoites. Plasmodium vivax of tertian is relatively easy to recognize, especially in a series of slides a few hours apart. The large ameboid forms, usual round gametocytes, the single chromatin dot and ring thickened on opposite side are points to be memorized. We are not impressed with the claim that by viewing small signet ring forms only, the species can be identified. It is true that falciparum has two dots and a uniform thin ring and may have two ring forms or more in a single cell. But P. vivax can at times emulate these characteristics. More important, as we have emphasized, would be the examination of a smear several hours later; if the rings have disappeared or remained the same, falciparum is present. If, however, the rings have thickened then vivax is the species. Another diagnostic point of value is the presence of ordinary Schuffner granules. These fine, reddish yellow dots are almost diagnostic of P. vivax infection. Of the fourth human Plasmodium, the ovale, we will say little since it is of no practical importance. It is remarkably similar to P. Malariae but has one very important characteristic: the infected erythrocyte is decidedly oval in shape.

Summary

The laboratory is of greatest value in diagnosis of malaria. Best of many procedures is the careful examination of thin as well as thick films. The novice will do well to emphasize thin films until experience with the thick drop is acquired. Classification of species is of some importance and the relative infrequency of mixed infections renders void any alibi in average cases. If the examiner entertains any doubts as to whether a structure is or is not a plasmodium, he will do very well by all concerned if he does not report malaria present. Once drugs are administered, there is decidedly less opportunity of finding Plasmodia.

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ABSTRACTS

CHRONIC LEUKEMIA: A STATISTICAL STUDY OF SYMPTOMS, DURATION OF LIFE AND PROGNOSIS: L. M. Pascucci, Rad., vol. 39, No. 1, July, '42, p. 75.

Of the series studied, 54% were men, with the myeloid cases evenly divided but the lymphatic more prevalent in the male than in the female. Symptoms, blood picture and treatment are summarized.

The author reports an average duration of life from onset of symptoms of 2.79 years for chronic myelogenous leukemia and 2.33 years for chronic lymphogenous leukemia. Roentgen therapy did not appear to prolong like in these cases but it did bring about remissions in which the patient's comfort was improved. Patients showing very low RBC and platelet counts at the first visit generally did not survive long. In general, the more chronic the disease, the longer is the life expectancy.

THE ANAEROBIC NONHEMOLYTIC STREPTOCOCCI IN SURGICAL INFECTIONS ON A GENERAL SURGICAL SERVICE: W. R. Sandusky, E. J. Pulaski, B. A. Johnson & F. L. Meleney, Surg., Gyn. & Obs., vol. 75, No. 2, Aug., '42, p. 145.

The literature on the anaerobic nonhemolytic streptococcus is briefly summarized. The authors report 170 cases of various surgical lesions from which this organism was isolated, in 29 of which it occurred in pure culture. These include perirectal abscess, infected pilonidal cyst and infected sebaceous cyst, sites which had not previously been reported.

These organisms are normally found in the mouth, intestine and female genital tract as saprophytes but apparently changing conditions vary their role. The authors emphasize the importance of anaerobic as well as aerobic investigation in all infections.

SHORTCOMINGS OF COMMERCIAL STEAM DISINFECTION APPARATUS: A. G. Gadd and A. W. Turner, Jr. Royal Army Med. Corps, vol. 78, No. 1, Apr., '42, p. 1.

Several models of steam sterilizers offered to the British War Department were tested using blankets as the typical load and using small tubes containing substances of known melting points to indicate efficiency. In general the efficiency was found to be surprisingly low. Specified maximum loads were usually found to be greater than the apparatus could carry successfully.

Materials should be packed loosely. The compactness of the load and the distance to its center were the most important factors.

The authors recommend using as many as 30 fusion tubes in making the original test on a sterilizer and in making the check after the structural defects have been corrected.

In drying after sterilizing, the air inlet was frequently found to be near the floor of the "dirty" preparation room and the air did not reach sterilization temperature before reaching the sterile contents of the apparatus. "Drying" for 30 minutes was not found effective for water removal.

ACTINOMYCOSIS OF THE TEMPORAL BONE: J. M. Brown, Laryngoscope, vol. LII, No. 7, July, '42, p. 507.

Dr. Brown describes the usual course of an actinomycotic lesion as roughly resembling a tubercle in its early stages but quickly undergoing suppuration with the production of fairly large abscess cavaties followed by fistulous tracts.

Diagnosis may be made by microscopic examination of the characteristic "sulphur granules" from fresh discharge. These, when crushed and freshly mounted in a basic solution show the following 3 zones: (1) a central zone of compact pigment, (2) an intermediate zone irregularly amorphous and deeply pigmented, (3) an outer zone of striated groups of mycelial hyphae.

Sulfanilamide has been reported as a cure. Of human actinomycosis, about 60% occur in the head and neck. They spread by fistulous tracts and multiple abscesses in the soft tissue and by an osteomyelitic process in the bone. To quote Dr. Brown, "any chronic progressive infection in the head and neck in which the cultures are consistently negative should arouse suspicion and a bacterial examination should be made for fungi."

THE PEPARATION OF LOEFFLER'S MEDIUM IN RELATION TO THE MORPHOLOGY OF C. DIPHTHERIAE: N. E. Goldsworthy and H. Wilson, Jr. Path. & Bact., vol. LIV No. 2, April, '42, p. 183.

Loeffler's medium prepared in 6 different laboratories was found to vary greatly in its ability to support growth of C. diphtheriae with classical morphology. Best results were obtained on media prepared from previously sterilized serum and glucose broth heated to inspissate only. Autoclaved media were relatively bad.

The authors suggest submitting this medium to the lowest temperature for the shortest time necessary to inspissate.

THE RELATION OF BLOOD GROUPS TO DRUG REACTIONS. A PRELIMINARY REPORT: H. H. Carroll, U. S. Nav. Med. Bull., vol. XL No. 3, July, '42, p. 598.

The author noticed that all of the 6 cases under his care with severe agranulocytosis following sulfanilamide therapy belonged to blood group A. He then made inquiry regarding the blood group of cases of severe sulfanilamide reactions reported in the literature. Group O was found to have less than half as many reactors as group A even though it is slightly more common. Of 13 fatalities reported, 1 was in AB, 10 in A, 0 in B and only 2 in 0.

While the number of cases surveyed is small, there seems to be some indication that the agglutinins and agglutinogens present in all groups except group O may be concerned in the susceptibility to reactions.

BLOOD FINDINGS IN CYCLOTRON WORKERS: S. Warren, Rad., vol. 39, No. 2, Aug., '42, p. 194.

This study concerns blood counts and hemoglobin determinations of 85 persons exposed to slight radiation from cyclotrons and radioactive substances. Barring other diseases, most persons under these conditions show no significant changes. Four showed minor variations in WBC. As in the case of exposure to X-ray and radium, depressions in WBC are followed by elevations. Marked fluctuations in WBC following slight exposure are due to instabilities of the bone marrow and persons of this type should not work where they are exposed to radiation.

BACTERIAL WARFARE: THE USE OF BIOLOGIC AGENTS IN WARFARE. Major L. A. Fox, Military Surgeon, vol. 90, No. 5, May, '42, p. 563.

The author presents the factors which would prevent successful use of organisms of the various types of transmission. He quotes the observations of the report of a group of scientists to the League of Nations, namely, (a) the effects of bacterial injury cannot be limited or localized, (b) modern water purification methods protect against the organisms of typhoid and cholera (c) plague is a disease that would be as dangerous for the force using the organisms as for the attacked (d) the danger from typhus has been exaggerated (e) modern sanitary methods are effective in controlling communicable diseases.

The development of epidemics depends largely upon resistance and sanitary and living conditions of the group. Since our populations are constantly exposed to these organisms without developing epidemics, the mere introduction of the organisms is not sufficient.

The introduction of agricultural pests is much more of a possibility but, since it usually takes years for these to spread over a large enough area to become effective, their use would be more likely to impoverish the conquered nation in the post-war years and thus cut down payment of indemnity to the victors.

FAMILIAL HEMOLYTIC JAUNDICE: A STUDY OF THE RESULTS OF SURGICAL THERAPY: C. W. McLaughlin, Jr., Surgery, vol. 12, No. 3, Sept., '42, p. 419.

Thirteen cases of familial hemolytic jaundice in which splenectomy effected clinical cure are presented. Transfusion before splenectomy was not practiced because a survey of the literature showed reactions as frequently as 45% of cases in spite of accurate crossmatching, with severe hemolysis of RBC and a further drop in the count.

All these patients showed an average increase in hemoglobin of 23.2% and in RBC of 1,100,000 during and in the hour following surgery with a gradual fall to pre-operative levels during the next 48 hours. The author suggests that this may result from "autotransfusion" or the outpouring of cells from an enlarged

splenic reservoir during surgical manipulation with the visible shrinkage of the spleen which is characteristic of splenectomy in familial hemolytic jaundice but does not occur in other splenectomies. Transfusion may follow splenectomy with favorable results but is usually not necessary in these cases.

Recurrence of the hemolytic factor may be due to a misdiagnosis, accessory splenic tissue not removed, or the assumption of a hemolytic character by hemolymph tissue.

THE OCCURRENCE OF TWO SIGNIFICANTLY DISTINCT RACES OF ENDAMOEBA HISTOLYTICA: J. J. Sapero, E. G. Hakansson & C. M. Louttit, Am. Jr. Trop. Med., vol. 22, No. 3, May, '42, p. 191.

A study of the dimensions of 7495 cysts from 320 cases showed they might be divided into a large strain and a small strain. Ten microns was used as the dividing line with 9 microns in wet-fixed stained cysts mounted in balsam.

Trophozoites of the small race show less motility and fewer ingested RBC than those of the large. One hundred attempts to culture the small race failed while in the large race, using the same method, 80% were positive.

STAPHYLOCOCCAL FOOD POISONING: G. G. Slocum, Sou. Med. Jr., vol. 35, No. 8, Aug., '42, p. 765.

Clinically staphylococcus food poisoning differs from that of the Salmonella type in that the incubation period ranges from 1-8 hours after consumption, usually 2-4 hours, while that of Salmonella is 6-24 hrs., usually more than 8 hrs. In Staphylococcal poisoning the acute stage lasts only 6-8 hours with the temperature usually normal or subnormal while in the Salmonella type the acute stage lasts longer and is usually accompanied by fever. In the former recovery is rapid, ordinarily taking about 24 hours while in the Salmonella type it is several days. Fatalities in Staphylococcal poisoning are very rare and in Salmonella average 1-2%.

The large majority of true bacterial food poisoning investigated by the Food and Drug Administration during the last eleven years covered by this report have been Staphylococcic with Salmonella definitely established as the causative agent in only one instance.

EFFECT OF THERAPEUTIC BLOOD LEVELS OF SULFANILAMIDE AND SULFADIAZINE ON WOUND HEALING: H. A. Zintel, D. B. Freshwater, J. D. Hardy, W. M. Harris, C. S. Neer & S. W. Robinson, Surgery, vol. 12, No. 2, Aug., '42, p. 242.

Tensile strength and histological studies were made of wounds in skin, fascia and stomach wall of dogs receiving sufficient sulfanilamide and sulfadiazine to maintain therapeutic blood levels. When compared with similar data for wounds on dogs not under chemotherapy, indications were that sulfanilamide and sulfadiazine do not retard healing at the concentration usually considered therapeutic.

CHRONIC BILIARY TYPHOID CARRIERS: F. A. Coller & P. Crabtree, Surgery, vol. 12, No. 3, Sept., '42, p. 426.

Of 21 biliary typhoid carriers undergoing cholecystectomy, 16 were definitely cured and 3 more were apparently cured. These cured cases may be divided into 2 groups, one in which the feces rapidly became negative and the other in which positive results were found for a much longer time.

Cholecystectomy is advocated as the best method of treating chronic typhoid carriers of the biliary type.

GAS GANGRENE: EXPERIMENTAL OBSERVATIONS ON THE USE OF THE SULFONAMIDE DERIVATIVES AND ZINC PEROXIDE IN ITS TREATMENT AND PREVENTION: G. A. Caldwell & F. J. Cox, Sou. Med. Jr., vol. 35, No. 9, Sept., '42.

These experiments were carried out on guinea pigs. Observations showed that surgical debridement was effective in preventing development and spread of gas gangrene. The inhibitory action of zinc peroxide and of the sulfonamide drugs when applied locally was demonstrated. The authors advocate the local implantation of sulfathiazole at the scene of the accident as a preventive measure.

ALKALOSIS AND GASTRIC TETANY DUE TO PROLONGED VOMITING AFTER A FINNEY PYLOROPLASTY: A. O. Wilensky, Rev. of Gastroenterology, vol. 9, No. 4, July-Aug., '42, p. 261.

The case presented was operated upon for a peptic ulcer in the duodenum with a moderate pyloric stenosis. This was followed by

gastric retention which was relieved by means of a Levin tube. Excessive drainage continued and the fluid loss was made up with 4,000-6,000 cc. 5% glucose in saline intravenously daily. On the 18th post-operative day muscular twitchings of the extremities occurred and the patient appeared to have developed an alkalosis tetany. CO_2 combining power was 97% and the blood chlorides fell to 260 mg.%

The explanation given was that continued loss of H ions in the vomitus had tended to produce alkalosis. This was aggravated by the loss of Cl ions which produced hypotonicity of the blood with the resultant substitution of CO_3 and HCO_3 ions. This in turn shifted the partition of diffusible blood calcium to the molecular state and brought about a tetany.

The administration of ammonium chloride brought about recovery with discharge 10 days later.

DIAGNOSIS OF GASTRIC DISORDERS BY THE EXCRETION OF DYES: C. L. Glaessner, Rev. of Gastroenterology, vol. 9, No. 4, July-Aug., '42, p. 269.

For this test 5 cc. of a 1% solution of neutral red were administered intramuscularly and gastric juice was collected every 2 min. to observe the appearance of the dye. It is secreted only by the acid-producing cells and bears no relationship to the secretion of pepsin. In cases of hyperacidity, the dye appeared in 5-9 min., in normal acidity in 10-20 min., and in hypoacidity in 20-35 min. True achylias showed no dye secretion even after hours. False achylias showed prolonged elimination.

RADIUM PROTECTION DURING AIR RAIDS: Radiology, vol. 39, No. 2, Aug., '42, p. 230.

This editorial discusses means of protection of radium during air raids as put forth in Handbook H 38, issued by the National Bureau of Standards and entitled "Protection of Radium During Air Raids." The precautions are advocated to decrease the hazards and danger to human life from radium dispersed by explosion rather than from a desire to prevent loss of radium. The handbook is available for 10c from the Superintendent of Documents, Washington, D. C.

BOOK REVIEWS

WAR MEDICINE, A Symposium. Editor, Winfield Scott Pugh, M.D., Commander, (M.C.) U.S.N. Retired, Formerly Surgeon, City Hospital, New York; Associate Editor, Edward Podolsky, M.D.; Technical Editor, Dagobert D. Runes, Ph.D. Publishers, The Philosophical Library, Inc., 15 East 40th Street, New York, N. Y. Publication date August 25, 1942. Price $7.50.

This symposium on War Medicine is a compact resumé of 57 subjects by 67 authors, physicians and surgeons in and out of the Armed Forces of the United States and Great Britain. Some of the work is published by courtesy of the Military Surgeon, official publication of the Association of Military Surgeons of the United States, The British Medical Journal, American Journal of Surgery, U. S. Naval Medical Bulletin, Surgery, Gynecology and Obstetrics and other general and special publications. The work is divided into three sections, namely, Surgery, Aviation and Naval Medicine and General Medicine. The subject matter has been boiled down to the essential facts. Recent advances in the treatment of war wounds, shock, use of the sulfonamides, plasma, etc., have been treated somewhat more in detail. Various substances such as vitamins, antiseptics, maggot extract, barberry, pectin, silicon, the influence of hypoprotemeia, etc., are briefly discussed in their relation to wound healing. War wounds of the brain, eyes, ears, heart, chest, abdomen and extremities are each discussed in separate chapters. Under Aviation and Naval Medicine the influences of various under-water and high altitude pressures and other problems peculiar to high flying and deep sea diving are outlined and treated in an all too brief manner. There still seem to be many problems yet to be solved from the physical and mental standpoints of the aviator particularly with the rapid advances made toward mechanical perfection and greatly increased speed of planes. Under the section on General Medicine foodstuffs for the soldier, allergy in Military Medicine, psychiatric problems, cardiovascular and other medical problems in relation to the armed forces are discussed. The book serves a very

useful purpose in offering a preliminary outline of medical practice under war conditions. It is very simply written and not highly technical. Some chapters have references or a bibliography. An index has been omitted.

FIRST AID. SURGICAL AND MEDICAL: By Warren H. Cole, M.D., F.A.C.S., Professor and Head of the Department of Surgery, University of Illinois College of Medicine; Director of Surgical Service, Illinois Research and Educational Hospitals, Chicago, and Charles B. Puestow, B.S., M.S., M.D., Ph.D., F.A.C.S., Associate Professor of Surgery, University of Illinois College of Medicine and Graduate School; Surgeon, Illinois Research and Educational Hospitals; Senior Surgeon, Henrotin Hospital; Consulting Surgeon, Municipal Contagious Disease Hospital and Bethany Home and Hospital, Chicago, and Saint James Hospital, Chicago Heights. D. Appleton-Century Company, Incorporated, New York and London, 1942, pp. 361, Price $3.00

First aid in its broadest sense presented by two eminent surgeons and teachers of surgery in collaboration with seventeen able and well known medical and surgical specialists.

The authors' prefatory comments arrested the reviewer's attention since he was curious to know the circumstances prompting this publication. There are two primary reasons: necessity born of the War to instruct medical students (and others) in first aid and a belief that such instruction should become a part of the medical curriculum even in time of peace. This excellent course is offered, therefore, for practical instruction with the injunction to all and sundry that First Aid "should be reserved for physicians when they are available . . . yet until a physician arrives . . . helpful . . . even life-saving . . . aid may be rendered by trained non-medical personnel."

The scope of this text is most comprehensive, the subject matter being divided into twenty-one sections, with a reference list and adequate index. Head injuries, abdominal injuries, respiratory emergencies, fractures, dislocations, frost-bite, urologic emergencies, gas and bomb raids, war wounds, burns, shock, transportation of the injured and various medical, in contrast to surgical, emergencies are covered clearly in this volume. Its simple terminology makes the book adaptable to use for instructing the layman but it is

sufficiently technical and scientific to be a valuable source of information for physicians and nurses as well. Original line drawings are used for illustrative purposes. The choice of type makes easy reading and main and minor subjects are so titled that they are not readily lost in the text. It is a good book on a pertinent subject.

A CURRICULUM FOR SCHOOLS OF MEDICAL TECHNOLOGY: By Israel Davidsohn, M.D., Director of Laboratories and Pathologist, Mount Sinai Hospital, Chicago, and Assistant Professor of Pathology, College of Medicine of the University of Illinois. Second Edition, Revised, Recommended by the Board of Registry of the American Society of Clinical Pathologists. 1942, pp. 47.

As the title informs this book is primarily intended for the use of those who need a guide in training medical laboratory workers. The first edition was published in 1937 under the title, "Model Curriculum for Teaching Students in Medical Technology." The current revision enlarges appreciably on the main theme of its predecessor offering information to prospective students as to "the realities of medical technology," describing eligibility and qualifications as established by the Registry and giving in specific outline the gradations of training required for this auxiliary branch of pathology. This book fulfills a definite purpose most admirably, being unique in its concept but sound in its precepts. It should be an invaluable outline for those responsible for training medical technologists and should prove equally as useful as a guide for instruction of medical students in this essential aspect of medicine.

NEWS AND ANNOUNCEMENTS

SCIENTIFIC SESSION OF THE AMERICAN ACADEMY OF PHYSICAL MEDICINE

Boston, October 14 to 17, 1942

The American Academy of Physical Medicine will hold its Twentieth Annual Scientific Session at the Hotel Statler, Boston, October 14 to 17, 1942. The program will be composed of clinical and scientific presentations involving techniques of importance in Wartime Medicine.

Topics of the discussions and demonstrations include Physical Medicine in relation to Aviation Medicine, Physical Education, Habilitation, Rehabilitation, First Aid, and War Injuries, as well as consideration of the use of physical agents in injury and disease falling within the scope of various medical specialties. There will be symposia on Poliomyelitis and Electrosurgery. Encephalography, Electroshock, Fever Therapy, and Other Special Procedures will be discussed by outstanding authorities.

Speakers will include pioneers in the development of physical medicine in the earlier World War, physicians actively concerned with military medicine, and leaders in the special medical fields. A clinic will be conducted at the Massachusetts General Hospital. The Academy will have the cooperation of the Massachusetts Institute of Technology and other Medical and Scientific Institutions.

Captain William Seaman Bainbridge, M.C., U.S.N., is the President. The Chairman of the Committee on Program is Lt. Col. William D. McFee, M.C., U.S.A.R. Physicians are invited to attend without registration fee. A copy of the official program can be obtained from the Secretary-Treasurer, Herman A. Osgood, M.D., 144 Commonwealth Avenue, Boston, Mass.

The Third Annual Medical Meeting of the National Foundation for Infantile Paralysis will be held in New York City on December 3-4, inclusive.

ABSTRACTS OF FOOD AND NUTRITION RESEARCH

Biological Abstracts announces the establishment of a new (seventh) section, "Specially Assembled Abstracts of Food and Nutrition Research" to be initiated in January, 1943. This section will consist of an assembly and reprinting of all the abstracts contained in Biological Abstracts that deal with human and animal nutrition and metabolism, vitamins, diet and diet-deficiency diseases, food composition and values, food processing and food microbiology, beverages, storage and conservation of foods, food spoilage and, in short, all of the biological literature that pertains to foods and nutrition.

Biological Abstracts has covered this literature ever since its establishment in 1926. In previous volumes the abstracts pertaining to foods and nutrition have been dispersed throughout the entire volume, hence research men and teachers whose special interests lay in the foods-nutrition field were able to obtain the abstracts pertaining to this literature only through the purchase of the complete edition or all of the five original sections. The segregation of the foods and nutrition abstracts into this new independently published section will provide, for the convenience of these workers and biologists generally, an abstracting service at greatly reduced cost.

In line with the general policies of Biological Abstracts every possible effort is being made to insure completeness in coverage of the world's literature. Efforts to obtain abstracts of publications from continental Europe, now mostly unavailable to workers in this country, are continuing. In spite of the very great restriction in diffusion of research information, occasioned by the war, journal coverage in Biological Abstracts has steadily increased until, at present, more than 1,700 periodicals in the biological field are being abstracted. The new abstract section will therefore, from the beginning, afford a very complete coverage of the world's research literature.

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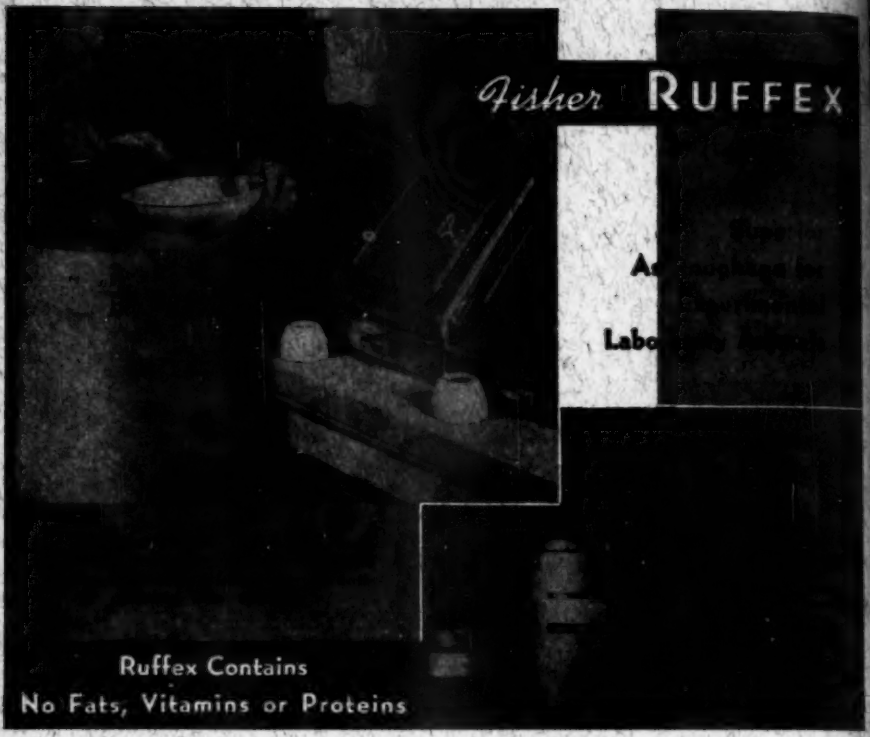

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